

THAT WHICH IS CLAIMED:

1. A method for transforming a freshly isolated, immature maize embryo with a nucleotide construct of interest, said method comprising:
 - (a) obtaining at least one immature embryo from a maize plant; and
 - (b) introducing said nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment.
2. The method of claim 1, further comprising contacting said immature embryo with an auxin-depleted transformation support medium prior to said bombardment.
3. The method of claim 1 wherein said immature embryo is obtained about 6 days to about 14 days after pollination.
4. The method of claim 2 wherein said auxin-depleted transformation support medium comprises a high concentration of an osmoticum.
5. The method of claim 4 wherein said auxin-depleted transformation support medium comprises an osmoticum consisting of sucrose, sorbitol, mannitol, polyethylene glycol, or combinations thereof.
6. The method of claim 2 wherein said auxin-depleted transformation support medium is phytohormone depleted.
7. The method of claim 1 wherein said microprojectile bombardment comprises low-velocity impact of at least one microprojectile with said immature embryo.

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8. The method of claim 7 wherein said microprojectile bombardment further comprises a gas pressure acceleration system comprising a rupture disk with a rupture disk rating that is at or below about 500 psi.

- 5 9. The method of claim 8 wherein said rupture disk rating comprises 100, 150,
200, 250, 300, 350, 400, 450 or 500 psi.

10. The method of claim 7 further comprising positioning said immature embryo between about 5 cm and about 12 cm from the macrocarrier platform.

11. The method of claim 2 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 6 hours before said nucleotide construct is introduced.

12. The method of claim 11 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 4 hours before said nucleotide construct is introduced.

13. The method of claim 12 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 2 hours before said nucleotide construct is introduced.

14. The method of claim 1 wherein the genotype of said immature embryo is Hi-II or a hybrid from a cross of Hi-II with a second genotype.

15. The method of claim 14 wherein said second genotype is PHN46, PHTE4, PHAA0, PHP18, PH05F, PH09B, PHP02, PHJ90, PH24E, PHT05, ASKC27 or PH21T.

16. The method of claim 1 further comprising regenerating said cell into a stably transformed maize plant.

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- (b) introducing a nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment; and
- (c) regenerating said cell into a transgenic maize plant wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.

26. The method of claim 25, further comprising contacting said immature embryo with an auxin-depleted transformation support medium prior to said bombardment.

27. The method of claim 25 wherein said immature embryo is obtained about 6 days to about 14 days after pollination.

28. The method of claim 26 wherein said auxin-depleted transformation support medium comprises a high concentration of an osmoticum.

29. The method of claim 28 wherein said auxin-depleted transformation support medium comprises an osmoticum of sucrose, sorbitol, mannitol, polyethylene glycol, or combinations thereof.

30. The method of claim 26 wherein said auxin-depleted transformation support medium is phytohormone depleted.

31. The method of claim 25 wherein said microprojectile bombardment comprises low-velocity impact of at least one microprojectile with said immature embryo.

32. The method of claim 31 wherein said microprojectile bombardment further comprises a gas pressure acceleration system comprising a rupture disk with a rupture disk rating that is at or below about 500 psi.

33. The method of claim 32 wherein said rupture disk rating comprises 100, 150, 200, 250, 300, 350, 400, 450 or 500 psi.
34. The method of claim 31 further comprising positioning said immature embryo between about 5 cm and about 12 cm from the macrocarrier platform.
35. The method of claim 26 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 6 hours before said nucleotide construct is introduced.
36. The method of claim 35 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 4 hours before said nucleotide construct is introduced.
37. The method of claim 36 wherein said immature embryo is held on said auxin-depleted transformation support medium not more than about 2 hours before said nucleotide construct is introduced.
38. The method of claim 25 wherein the genotype of said immature embryo is Hi-II or a hybrid from a cross of Hi-II with a second genotype.
39. The method of claim 38 wherein said second genotype is PHN46, PHTe4, PHAA0, PHP18, PH05F, PH09B, PHP02, PHJ90, PH24E, PHT05, ASKC27 or PH21T.
40. The method of claim 25 wherein said nucleotide construct comprises a selectable marker gene, a marker gene or a cell cycle gene.
41. The method of claim 40 wherein said marker gene comprises a selectable marker gene.

42. The method of claim 41 wherein said selectable marker gene is *bar*, *nptII*, *hpt*, the moCAH gene, herbicide resistance genes or antibiotic resistance genes.

43. The method of claim 40 wherein said marker gene comprises GFP.

44. The method of claim 40 wherein said regenerating comprises selection for the expression of said selectable marker gene.

45. The method of claim 40 where said nucleotide construct further comprises at least one nucleotide sequence of interest.

46. The method of claim 25 wherein said regenerating comprises inducing somatic embryogenesis.

47. The method of claim 46 wherein said somatic embryogenesis comprises providing said cell with an effective amount of an auxin.

48. The method of claim 47 wherein said auxin comprises 2,4-dichlorophenoxyacetate (2,4-D), indoleacetic acid (IAA), 3-indolebutyric acid (IBA), α -naphthaleneacetic acid (NAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), (4-chloro-2-methylphenoxy)acetic acid (MCPA), dicamba, chloramben or combinations thereof.

49. A method for introducing a nucleotide construct into at least one cell within a twenty-four hour period of time comprising:

- (a) obtaining at least one immature embryo from a maize plant; and
- (b) introducing a nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment.

	Mean (SD)	Median (IQR)	Range	Skewness	Kurtosis	Shapiro-Wilk	Normality
Age	65.4 (10.2)	64 (54-72)	45-85	0.12	-0.05	0.98	Normal
Gender	Male (65.4%)	Male (65.4%)	Male (65.4%)	0.12	-0.05	0.98	Normal
Education	12.5 (2.1)	12 (10-14)	8-16	0.12	-0.05	0.98	Normal
Income	15.2 (3.5)	14 (12-16)	10-20	0.12	-0.05	0.98	Normal
Health	78.5 (12.1)	75 (65-85)	50-95	0.12	-0.05	0.98	Normal
Activity	12.5 (2.1)	12 (10-14)	8-16	0.12	-0.05	0.98	Normal
Stress	15.2 (3.5)	14 (12-16)	10-20	0.12	-0.05	0.98	Normal
Quality of Life	78.5 (12.1)	75 (65-85)	50-95	0.12	-0.05	0.98	Normal

50. The method of Claim 49 further comprising contacting said immature embryo with an auxin-depleted transformation support medium for not more than 12 hours prior to said bombardment.

- 5 51. The method of Claim 49 further comprising regenerating said cell into a transgenic maize plant wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.

52. The method of Claim 50 wherein said immature embryo is held on said auxin-
depleted transformation support medium for not more than about 6 hours
before said nucleotide construct is introduced.

53. The method of Claim 52 wherein said immature embryo is held on said auxin-depleted transformation support medium for not more than about 4 hours before said nucleotide construct is introduced.

54. The method of Claim 50 further comprising a high concentration of an osmoticum.

- 20 55. A method for high frequency stable transformation of freshly excised embryos
said method comprising:

- (a) obtaining at least one immature embryo from a maize plant;
- (b) introducing said nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment comprising 0.6 μ Au particles, rupture disk rating of about 200, and positioning said immature embryo between about 8 cm and about 12 cm from the macrocarrier platform.

56. The method of Claim 55 further comprising contacting said immature embryo
30 with an auxin-depleted transformation support medium prior to said
bombardment.

57. The method of Claim 56 wherein the auxin-depleted transformation support medium comprises about 12% to about 19% sucrose.

- 5 58. A method for improving transformation frequency compared to precultured controls comprising;
- (a) obtaining at least one immature embryo from a maize plant; and
 - (b) introducing a nucleotide construct into at least one cell of said immature embryo by microprojectile bombardment.

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59. The method of Claim 58 further comprising contacting said immature embryo with an auxin-depleted transformation support medium prior to said bombardment.

15 60. The method of Claim 59 wherein the auxin-depleted transformation support medium comprises about 12% to about 19% sucrose.

20 61. The method of Claim 60 wherein introducing said microprojectile bombardment comprises 0.6 μ Au particles, rupture disk rating of about 200, and positioning said immature embryo between about 8 cm and about 12 cm from the macrocarrier platform.

25 62. The method of Claim 58 further comprising regenerating said cell into a transgenic maize plant wherein said cell comprises stably incorporated in its genome at least one copy of said nucleotide construct or part thereof.